

CLAIMS

1. A nitrile hydratase comprising an  $\alpha$ -subunit and a  $\beta$ -subunit, wherein the  $\alpha$ -subunit has an amino acid sequence in which at least one amino acid of the 36<sup>th</sup>, 71<sup>st</sup>, 148<sup>th</sup> and 204<sup>th</sup> amino acids in the amino acid sequence of SEQ ID NO: 1 in the Sequence Listing is substituted by another amino acid.
2. The nitrile hydratase according to claim 1, wherein at least one amino acid of the 6<sup>th</sup>, 19<sup>th</sup>, 38<sup>th</sup>, 77<sup>th</sup>, 90<sup>th</sup>, 102<sup>nd</sup>, 106<sup>th</sup>, 126<sup>th</sup>, 130<sup>th</sup>, 142<sup>nd</sup>, 146<sup>th</sup>, 187<sup>th</sup>, 194<sup>th</sup> and 203<sup>rd</sup> amino acids in the amino acid sequence of the  $\alpha$ -subunit is further substituted by another amino acid.
3. The nitrile hydratase according to claim 1 or 2, wherein the  $\beta$ -subunit has the amino acid sequence of SEQ ID NO: 2 in the Sequence Listing.
4. The nitrile hydratase according to claim 3, wherein at least one amino acid of the 10<sup>th</sup>, 32<sup>nd</sup>, 37<sup>th</sup>, 41<sup>st</sup>, 46<sup>th</sup>, 48<sup>th</sup>, 51<sup>st</sup>, 72<sup>nd</sup>, 118<sup>th</sup>, 127<sup>th</sup>, 146<sup>th</sup>, 160<sup>th</sup>, 186<sup>th</sup> and 217<sup>th</sup> amino acids in the amino acid sequence of the  $\beta$ -subunit is further substituted by another amino acid.
5. The nitrile hydratase according to claim 4, wherein

at least one amino acid of the 20<sup>th</sup>, 21<sup>st</sup>, 108<sup>th</sup>, 200<sup>th</sup> and 212<sup>th</sup> amino acids in the amino acid sequence of the  $\beta$ -subunit is further substituted by another amino acid.

5       6. The nitrile hydratase according to any one of claims 1 to 5, wherein at sites other than the amino acid substitution sites in the amino acid sequence carried by at least one of the  $\beta$ -subunit and the  $\alpha$ -subunit, one or several amino acids are subject to substitution, insertion or  
10 deletion within the scope of not impairing the nitrile hydratase activity.

7. A nitrile hydratase comprising an  $\alpha$ -subunit and a  $\beta$ -subunit, wherein the  $\beta$ -subunit has an amino acid sequence 15 in which at least one amino acid of the 10<sup>th</sup>, 32<sup>nd</sup>, 37<sup>th</sup>, 41<sup>st</sup>, 46<sup>th</sup>, 48<sup>th</sup>, 51<sup>st</sup>, 72<sup>nd</sup>, 118<sup>th</sup>, 127<sup>th</sup>, 146<sup>th</sup>, 160<sup>th</sup>, 186<sup>th</sup> and 217<sup>th</sup> amino acids in the amino acid sequence of SEQ ID NO: 2 in the Sequence Listing is substituted by another amino acid.

20       8. The nitrile hydratase according to claim 7, wherein at least one amino acid of the 20<sup>th</sup>, 21<sup>st</sup>, 108<sup>th</sup>, 200<sup>th</sup> and 212<sup>th</sup> amino acids in the amino acid sequence of the  $\beta$ -subunit is further substituted by another amino acid.

25       9. The nitrile hydratase according to claim 7 or 8,

wherein the  $\alpha$ -subunit has the amino acid sequence of SEQ ID NO: 1 in the Sequence Listing.

10. The nitrile hydratase according to claim 9,  
5 wherein at least one amino acid of the 36<sup>th</sup>, 71<sup>st</sup>, 148<sup>th</sup> and  
204<sup>th</sup> amino acids of the  $\alpha$ -subunit is substituted by another  
amino acid.

11. The nitrile hydratase according to claim 10,  
10 wherein at least one amino acid of the 6<sup>th</sup>, 19<sup>th</sup>, 38<sup>th</sup>, 77<sup>th</sup>,  
90<sup>th</sup>, 102<sup>nd</sup>, 106<sup>th</sup>, 126<sup>th</sup>, 130<sup>th</sup>, 142<sup>nd</sup>, 146<sup>th</sup>, 187<sup>th</sup>, 194<sup>th</sup> and  
203<sup>rd</sup> amino acids in the amino acid sequence of the  $\alpha$ -subunit  
is further substituted by another amino acid.

15 12. The nitrile hydratase according to any one of  
claims 7 to 11, wherein at sites other than the amino acid  
substitution sites in the amino acid sequence carried by at  
least one of the  $\beta$ -subunit and the  $\alpha$ -subunit, one or several  
amino acids are subject to substitution, insertion or  
20 deletion within the scope of not impairing the nitrile  
hydratase activity.

13. A gene encoding the amino acid sequence of the  $\alpha$ -  
subunit of nitrile hydratase, wherein the amino acid  
25 sequence is an amino acid sequence in which at least one

amino acid of the 36<sup>th</sup>, 71<sup>st</sup>, 148<sup>th</sup> and 204<sup>th</sup> amino acids in the amino acid sequence of SEQ ID NO: 1 in the Sequence Listing is substituted by another amino acid.

5 14. The gene according to claim 13, wherein at least one amino acid of the 6<sup>th</sup>, 19<sup>th</sup>, 38<sup>th</sup>, 77<sup>th</sup>, 90<sup>th</sup>, 102<sup>nd</sup>, 106<sup>th</sup>, 126<sup>th</sup>, 130<sup>th</sup>, 142<sup>nd</sup>, 146<sup>th</sup>, 187<sup>th</sup>, 194<sup>th</sup> and 203<sup>rd</sup> amino acids in the amino acid sequence is further substituted by another amino acid.

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15 15. The gene according to claim 13 or 14, wherein at sites other than the amino acid substitution sites carried by the amino acid sequence of the  $\alpha$ -subunit, one or several amino acids are subject to substitution, insertion or 15 deletion within the scope of not impairing the nitrile hydratase activity.

16. The gene according to claim 13, wherein it has a base sequence obtained by substituting at least one base 20 sequence among the 106<sup>th</sup> to 108<sup>th</sup>, 211<sup>th</sup> to 213<sup>th</sup>, 442<sup>nd</sup> to 444<sup>th</sup>, and 610<sup>th</sup> to 612<sup>th</sup> of the base sequence of SEQ ID NO: 3 in the Sequence Listing with another base sequence.

17. The gene according to claim 16, wherein at least 25 one base sequence among the 16<sup>th</sup> to 18<sup>th</sup>, 55<sup>th</sup> to 57<sup>th</sup>, 112<sup>th</sup>

to 114<sup>th</sup>, 229<sup>th</sup> to 231<sup>st</sup>, 268<sup>th</sup> to 270<sup>th</sup>, 304<sup>th</sup> to 306<sup>th</sup>, 316<sup>th</sup> to 318<sup>th</sup>, 376<sup>th</sup> to 378<sup>th</sup>, 388<sup>th</sup>, to 390<sup>th</sup>, 424<sup>th</sup> to 426<sup>th</sup>, 436<sup>th</sup> to 438<sup>th</sup>, 559<sup>th</sup> to 561<sup>st</sup>, 580<sup>th</sup> to 582<sup>nd</sup>, and 607<sup>th</sup> to 609<sup>th</sup> of the base sequence is further substituted by another base

5 sequence.

18. A gene encoding the amino acid sequence of the  $\beta$ -subunit of nitrile hydratase, wherein the amino acid sequence is an amino acid sequence in which at least one 10 amino acid of the 10<sup>th</sup>, 32<sup>nd</sup>, 37<sup>th</sup>, 41<sup>st</sup>, 46<sup>th</sup>, 48<sup>th</sup>, 51<sup>st</sup>, 72<sup>nd</sup>, 118<sup>th</sup>, 127<sup>th</sup>, 146<sup>th</sup>, 160<sup>th</sup>, 186<sup>th</sup> and 217<sup>th</sup> amino acids in the amino acid sequence of SEQ ID NO: 2 in the Sequence Listing is substituted by another amino acid.

15 19. The gene according to claim 18, wherein at least one amino acid of the 20<sup>th</sup>, 21<sup>st</sup>, 108<sup>th</sup>, 200<sup>th</sup> and 212<sup>th</sup> amino acids in the amino acid sequence is further substituted by another amino acid.

20 20. The gene according to claim 18 or 19, wherein at sites other than the amino acid substitution sites carried by the amino acid sequence of the  $\beta$ -subunit, one or several amino acids are subject to substitution, insertion or deletion within the scope of not impairing the nitrile 25 hydratase activity.

21. The gene according to claim 18, having a base sequence which is obtained by substituting at least one base sequence among the 28<sup>th</sup> to 30<sup>th</sup>, 94<sup>th</sup> to 96<sup>th</sup>, 109<sup>th</sup> to 111<sup>th</sup>, 5 121<sup>st</sup> to 123<sup>rd</sup>, 136<sup>th</sup> to 138<sup>th</sup>, 142<sup>nd</sup> to 144<sup>th</sup>, 151<sup>st</sup> to 153<sup>rd</sup>, 214<sup>th</sup> to 216<sup>th</sup>, 352<sup>nd</sup> to 354<sup>th</sup>, 379<sup>th</sup> to 381<sup>st</sup>, 436<sup>th</sup> to 438<sup>th</sup>, 478<sup>th</sup> to 480<sup>th</sup>, 556<sup>th</sup> to 558<sup>th</sup>, and 649<sup>th</sup> to 651<sup>st</sup> of the base sequence as set forth in SEQ ID NO: 4 in the Sequence Listing with another base sequence.

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22. The gene according to claim 21, wherein at least one base sequence among the 58<sup>th</sup> to 60<sup>th</sup>, 61<sup>st</sup> to 63<sup>rd</sup>, 322<sup>nd</sup> to 324<sup>th</sup>, 598<sup>th</sup> to 600<sup>th</sup>, and 634<sup>th</sup> to 636<sup>th</sup> of the base sequence is further substituted by another base sequence.

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23. A gene encoding the nitrile hydratase, comprising the gene which codes for the amino acid sequence of the  $\alpha$ -subunit and the gene which codes for the amino acid sequence of the  $\beta$ -subunit of nitrile hydratase, wherein the amino acid sequence of the  $\alpha$ -subunit is an amino acid sequence in which at least one amino acid of the 36<sup>th</sup>, 71<sup>st</sup>, 148<sup>th</sup> and 204<sup>th</sup> amino acids in the amino acid sequence of SEQ ID NO: 1 in the Sequence Listing is substituted by another amino acid.

25 24. The gene according to claim 23, wherein at least

one amino acid of the 6<sup>th</sup>, 19<sup>th</sup>, 38<sup>th</sup>, 77<sup>th</sup>, 90<sup>th</sup>, 102<sup>nd</sup>, 106<sup>th</sup>, 126<sup>th</sup>, 130<sup>th</sup>, 142<sup>nd</sup>, 146<sup>th</sup>, 187<sup>th</sup>, 194<sup>th</sup> and 203<sup>rd</sup> amino acids in the amino acid sequence of the  $\alpha$ -subunit is further substituted by another amino acid.

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25. The gene according to claim 23 or 24, wherein at sites other than the amino acid substitution sites carried by the amino acid sequence of the  $\alpha$ -subunit, one or several amino acids are subject to substitution, insertion or 10 deletion within the scope of not impairing the nitrile hydratase activity.

26. The gene according to claim 23, wherein the gene encoding the amino acid sequence of the  $\alpha$ -subunit has a base sequence obtained by substituting at least one base sequence among the 106<sup>th</sup> to 108<sup>th</sup>, 211<sup>th</sup> to 213<sup>th</sup>, 442<sup>nd</sup> to 444<sup>th</sup>, and 610<sup>th</sup> to 612<sup>th</sup> of the base sequence of SEQ ID NO: 3 in the Sequence Listing with another base sequence.

20 27. The gene according to claim 26, wherein at least one base sequence among the 16<sup>th</sup> to 18<sup>th</sup>, 55<sup>th</sup> to 57<sup>th</sup>, 112<sup>th</sup> to 114<sup>th</sup>, 229<sup>th</sup> to 231<sup>st</sup>, 268<sup>th</sup> to 270<sup>th</sup>, 304<sup>th</sup> to 306<sup>th</sup>, 316<sup>th</sup> to 318<sup>th</sup>, 376<sup>th</sup> to 378<sup>th</sup>, 388<sup>th</sup> 390<sup>th</sup>, 424<sup>th</sup> to 426<sup>th</sup>, 436<sup>th</sup> to 438<sup>th</sup>, 559<sup>th</sup> to 561<sup>st</sup>, 580<sup>th</sup> to 582<sup>nd</sup>, and 607<sup>th</sup> to 609<sup>th</sup> of the 25 base sequence is further substituted by another base

sequence.

28. The gene according to any one of claims 23 to 27,  
wherein the amino acid sequence of the  $\beta$ -subunit is the  
5 amino acid sequence of SEQ ID NO: 2 in the Sequence Listing.

29. The gene according to any one of claims 23 to 27,  
wherein the amino acid sequence of the  $\beta$ -subunit is an amino  
acid sequence in which at least one amino acid of the 10<sup>th</sup>,  
10 32<sup>nd</sup>, 37<sup>th</sup>, 41<sup>st</sup>, 46<sup>th</sup>, 48<sup>th</sup>, 51<sup>st</sup>, 72<sup>nd</sup>, 118<sup>th</sup>, 127<sup>th</sup>, 146<sup>th</sup>,  
160<sup>th</sup>, 186<sup>th</sup> and 217<sup>th</sup> amino acids in the amino acid sequence  
of SEQ ID NO: 2 is substituted by another amino acid.

30. The gene according to claim 29, wherein at least  
15 one amino acid of the 20<sup>th</sup>, 21<sup>st</sup>, 108<sup>th</sup>, 200<sup>th</sup> and 212<sup>th</sup> amino  
acids in the amino acid sequence of the  $\beta$ -subunit is further  
substituted by another amino acid.

31. The gene according to claim 29 or 30, wherein at  
20 sites other than the amino acid substitution sites carried  
by the amino acid sequence of the  $\beta$ -subunit, one or several  
amino acids are subject to substitution, insertion or  
deletion within the scope of not impairing the nitrile  
hydratase activity.

32. The gene according to claim 28, wherein the gene encoding the amino acid sequence of the  $\beta$ -subunit has the base sequence of SEQ ID NO: 4 in the Sequence Listing.

5 33. The gene according to claim 29, wherein the gene encoding the amino acid sequence of the  $\beta$ -subunit has a base sequence obtained by substituting at least one base sequence among the 28<sup>th</sup> to 30<sup>th</sup>, 94<sup>th</sup> to 96<sup>th</sup>, 109<sup>th</sup> to 111<sup>th</sup>, 121<sup>st</sup> to 123<sup>rd</sup>, 136<sup>th</sup> to 138<sup>th</sup>, 142<sup>nd</sup> to 144<sup>th</sup>, 151<sup>st</sup> to 153<sup>rd</sup>, 214<sup>th</sup> to 216<sup>th</sup>, 352<sup>nd</sup> to 354<sup>th</sup>, 379<sup>th</sup> to 381<sup>st</sup>, 436<sup>th</sup> to 438<sup>th</sup>, 478<sup>th</sup> to 480<sup>th</sup>, 556<sup>th</sup> to 558<sup>th</sup>, and 649<sup>th</sup> to 651<sup>st</sup> of the base sequence as set forth in SEQ ID NO: 4 in the Sequence Listing with another base sequence.

15 34. The gene according to claim 33, wherein at least one base sequence among the 58<sup>th</sup> to 60<sup>th</sup>, 61<sup>st</sup> to 63<sup>rd</sup>, 322<sup>nd</sup> to 324<sup>th</sup>, 598<sup>th</sup> to 600<sup>th</sup>, and 634<sup>th</sup> to 636<sup>th</sup> of the base sequence is further substituted by another base sequence.

20 35. A gene encoding the nitrile hydratase, comprising the gene which codes for the amino acid sequence of the  $\alpha$ -subunit and the gene which codes for the amino acid sequence of the  $\beta$ -subunit of nitrile hydratase, wherein the amino acid sequence of the  $\beta$ -subunit is an amino acid sequence in which at least one amino acid of the 10<sup>th</sup>, 32<sup>nd</sup>, 37<sup>th</sup>, 41<sup>st</sup>,

46<sup>th</sup>, 48<sup>th</sup>, 51<sup>st</sup>, 72<sup>nd</sup>, 118<sup>th</sup>, 127<sup>th</sup>, 146<sup>th</sup>, 160<sup>th</sup>, 186<sup>th</sup> and 217<sup>th</sup> amino acids in the amino acid sequence of SEQ ID NO: 2 in the Sequence Listing is substituted by another amino acid.

5 36. The gene according to claim 35, wherein at least one amino acid of the 20<sup>th</sup>, 21<sup>st</sup>, 108<sup>th</sup>, 200<sup>th</sup> and 212<sup>th</sup> amino acids in the amino acid sequence is further substituted by another amino acid.

10 37. The gene according to claim 35 or 36, wherein at sites other than the amino acid substitution sites carried by the amino acid sequence of the  $\beta$ -subunit, one or several amino acids are subject to substitution, insertion or deletion within the scope of not impairing the nitrile 15 hydratase activity.

38. The gene according to claim 35, wherein the base sequence encoding the amino acid sequence of the  $\beta$ -subunit has a base sequence obtained by substituting at least one 20 base sequence among the 28<sup>th</sup> to 30<sup>th</sup>, 94<sup>th</sup> to 96<sup>th</sup>, 109<sup>th</sup> to 111<sup>th</sup>, 121<sup>st</sup> to 123<sup>rd</sup>, 136<sup>th</sup> to 138<sup>th</sup>, 142<sup>nd</sup> to 144<sup>th</sup>, 151<sup>st</sup> to 153<sup>rd</sup>, 214<sup>th</sup> to 216<sup>th</sup>, 352<sup>nd</sup> to 354<sup>th</sup>, 379<sup>th</sup> to 381<sup>st</sup>, 436<sup>th</sup> to 438<sup>th</sup>, 478<sup>th</sup> to 480<sup>th</sup>, 556<sup>th</sup> to 558<sup>th</sup>, and 649<sup>th</sup> to 651<sup>st</sup> of the 25 base sequence as set forth in SEQ ID NO: 4 in the Sequence Listing with another base sequence.

39. The gene according to claim 38, wherein at least one base sequence among the 58<sup>th</sup> to 60<sup>th</sup>, 61<sup>st</sup> to 63<sup>rd</sup>, 322<sup>nd</sup> to 324<sup>th</sup>, 598<sup>th</sup> to 600<sup>th</sup>, and 634<sup>th</sup> to 636<sup>th</sup> of the base sequence is further substituted by another base sequence.

40. The gene according to any one of claims 35 to 39, wherein the amino acid sequence of the  $\alpha$ -subunit has the amino acid sequence of SEQ ID NO: 1 in the Sequence Listing.

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41. The gene according to any one of claims 35 to 39, wherein the amino acid sequence of the  $\alpha$ -subunit is an amino acid sequence in which at least one amino acid of the 36<sup>th</sup>, 71<sup>st</sup>, 148<sup>th</sup> and 204<sup>th</sup> amino acids in the amino acid sequence of SEQ ID NO: 1 in the Sequence Listing.

42. The gene according to claim 41, wherein at least one amino acid of the 6<sup>th</sup>, 19<sup>th</sup>, 38<sup>th</sup>, 77<sup>th</sup>, 90<sup>th</sup>, 102<sup>nd</sup>, 106<sup>th</sup>, 126<sup>th</sup>, 130<sup>th</sup>, 142<sup>nd</sup>, 146<sup>th</sup>, 187<sup>th</sup>, 194<sup>th</sup> and 203<sup>rd</sup> amino acids in the amino acid sequence of the  $\alpha$ -subunit is further substituted by another amino acid.

43. The gene according to claim 41 or 42, wherein at sites other than the amino acid substitution sites carried by the amino acid sequence of the  $\alpha$ -subunit, one or several

amino acids are subject to substitution, insertion or deletion within the scope of not impairing the nitrile hydratase activity.

5        44. The gene according to claim 40, wherein the gene encoding the amino acid sequence of the  $\alpha$ -subunit has the base sequence of SEQ ID NO: 3 in the Sequence Listing.

10       45. The gene according to claim 41, wherein the gene encoding the amino acid sequence of the  $\alpha$ -subunit has a base sequence obtained by substituting at least one base sequence among the 106<sup>th</sup> to 108<sup>th</sup>, 211<sup>th</sup> to 213<sup>th</sup>, 442<sup>nd</sup> to 444<sup>th</sup>, and 610<sup>th</sup> to 612<sup>th</sup> of the base sequence of SEQ ID NO: 3 in the Sequence Listing with another base sequence.

15       46. The gene according to claim 45, wherein at least one base sequence among the 16<sup>th</sup> to 18<sup>th</sup>, 55<sup>th</sup> to 57<sup>th</sup>, 112<sup>th</sup> to 114<sup>th</sup>, 229<sup>th</sup> to 231<sup>st</sup>, 268<sup>th</sup> to 270<sup>th</sup>, 304<sup>th</sup> to 306<sup>th</sup>, 316<sup>th</sup> to 318<sup>th</sup>, 376<sup>th</sup> to 378<sup>th</sup>, 388<sup>th</sup> to 390<sup>th</sup>, 424<sup>th</sup> to 426<sup>th</sup>, 436<sup>th</sup> to 438<sup>th</sup>, 559<sup>th</sup> to 561<sup>st</sup>, 580<sup>th</sup> to 582<sup>nd</sup>, and 607<sup>th</sup> to 609<sup>th</sup> of the base sequence is further substituted by another base sequence.

20       47. A plasmid having the gene encoding the nitrile hydratase according to any one of claims 1 to 12.

48. The plasmid according to claim 47, wherein the gene encoding the  $\alpha$ -subunit of the nitrile hydratase is the base sequence of SEQ ID NO: 3 or the gene according to any 5 one of claims 13 to 17.

49. The plasmid according to claim 47 or 48, wherein the gene encoding the  $\beta$ -subunit of the nitrile hydratase is the base sequence of SEQ ID NO: 4 or the gene according to 10 any one of claims 18 to 22.

50. A plasmid having the gene encoding the nitrile hydratase according to any one of claims 23 to 46.

15 51. The plasmid according to any one of claims 47 to 50, which comprises the constitution for the expression of the nitrile hydratase in a host cell.

20 52. A transformant obtained by transforming a host cell with the plasmid according to claim 51.

53. A method for production of nitrile hydratase, comprising a step of cultivating the transformant according to claim 52 in a culture medium and producing a nitrile 25 hydratase based on the nitrile hydratase gene carried by the

plasmid in the transformant.

54. The method for production according to claim 53,  
further comprising a step of recovering nitrile hydratase  
5 from the transformant after the cultivation, the culture and  
a product of processing them.

55. A method for production of nitrile compound by  
bringing a nitrile compound into contact with a nitrile  
10 hydratase in an aqueous medium to obtain a corresponding  
nitrile compound, wherein the nitrile hydratase is one  
according to any one of claims 1 to 12.

56. A method for modifying an enzyme having the  
15 nitrile hydratase activity, which comprises changing one or  
more properties selected from the enzymatic activity,  
substrate specificity,  $V_{max}$ ,  $K_m$ , thermal stability, stability  
against the substrate and stability against the product, by  
specifying certain amino acid residues and performing  
20 substitution, insertion or deletion at one or more sites of  
the specified amino acid residues according to the following  
procedure:

(a) aligning the amino acid sequence of the enzyme  
having the nitrile hydratase activity before modification,  
25 with the amino acid sequence as set forth in SEQ ID NO: 98

in the Sequence Listing and the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing,

(b) specifying, based on the results of the alignment, the amino acid residues corresponding to the region 5 extending from the 36<sup>th</sup> threonine to the 48<sup>th</sup> asparagine in the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing, and to the region extending from the 31<sup>st</sup> lysine to the 51<sup>st</sup> phenylalanine and to the region extending from the 112<sup>th</sup> lysine to the 127<sup>th</sup> leucine in the amino acid 10 sequence as set forth in SEQ ID NO: 99 in the Sequence Listing, and

(c) performing substitution, insertion or deletion at one or more sites of the specified amino acid residues.

15 57. A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, substrate specificity,  $V_{max}$ ,  $K_m$ , thermal stability, stability against the substrate and stability against the product, by 20 specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

(d) aligning the amino acid sequence of the enzyme 25 having the nitrile hydratase activity before modification,

with the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing and the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing,

(e) specifying, based on the results of the alignment,  
5 the amino acid residues corresponding to the 36<sup>th</sup>, 48<sup>th</sup>, 71<sup>st</sup>, 148<sup>th</sup>, 188<sup>th</sup> and 204<sup>th</sup> of the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing, and of the amino acid residues corresponding to the 10<sup>th</sup>, 32<sup>nd</sup>, 33<sup>rd</sup>, 37<sup>th</sup>, 40<sup>th</sup>, 41<sup>st</sup>, 46<sup>th</sup>, 48<sup>th</sup>, 51<sup>st</sup>, 61<sup>st</sup>, 72<sup>nd</sup>, 112<sup>th</sup>, 118<sup>th</sup>, 127<sup>th</sup>, 146<sup>th</sup>,  
10 150<sup>th</sup>, 160<sup>th</sup>, 168<sup>th</sup>, 171<sup>st</sup>, 176<sup>th</sup>, 186<sup>th</sup>, 217<sup>th</sup> and 218<sup>th</sup> of the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing, and

(f) performing substitution, insertion or deletion at one or more sites of the specified amino acid residues.

15 58. A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, substrate specificity,  $V_{max}$ ,  $K_m$ , thermal stability, stability against the substrate and stability against the product, by  
20 specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

(g) inferring the stereostructure of the enzyme having  
25 the nitrile hydratase activity before modification by

carrying out an alignment based on the nitrile hydratase stereostructure and the amino acid sequence as set forth in PDB (Protein Data Bank) ID NO: 1IRE,

(h) specifying, based on the stereostructure inferred,  
5 the amino acid residues in the regions corresponding to the 2<sup>nd</sup> helix as counted from the N-terminal in Chain 1IRE: A, and to the 1<sup>st</sup> helix and the 2<sup>nd</sup> helix from the N-terminal, the loop portions inserted in the latter helices and the 3<sup>rd</sup> helix as counted from the C-terminal in Chain 1IRE: B in the  
10 nitrile hydratase stereostructure as set forth in PDB ID NO: 1IRE, and

(i) performing substitution, insertion or deletion at one or more sites of the specified amino acid residues.

15 59. A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, substrate specificity,  $V_{max}$ ,  $K_m$ , thermal stability, stability against the substrate and stability against the product, by  
20 specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

(j) inferring the stereostructure of the enzyme having  
25 the nitrile hydratase activity before modification by

carrying out an alignment based on the nitrile hydratase stereostructure and the amino acid sequence as set forth in PDB ID NO: 1IRE,

(k) specifying, based on the stereostructure inferred, 5 the four amino acid residues such as the amino acid residues which correspond to the 89<sup>th</sup> amino acid residue glutamine and the 165<sup>th</sup> amino acid residue glutamic acid as counted from the N-terminal in Chain A, and the amino acid residues which correspond to the 37<sup>th</sup> amino acid residue phenylalanine and 10 the 48<sup>th</sup> amino acid leucine as counted from the N-terminal in Chain B in the nitrile hydratase stereostructure as set forth in PDB ID NO: 1IRE,

(l) specifying the amino acid residues whose side-chain front-end heavy atoms are located within 5Å of radius in the 15 respective stereostructures having each of the side-chain front-end heavy atoms of the four above-specified amino acid residues as the point center, and

(m) performing substitution, insertion or deletion at one or more of the amino acid residues specified in the 20 above (l).

60. A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, 25 substrate specificity,  $V_{max}$ ,  $K_m$ , thermal stability, stability

against the substrate and stability against the product, by specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following 5 procedure:

- (n) inferring the stereostructure of the enzyme having the nitrile hydratase activity before modification by carrying out an alignment based on the nitrile hydratase stereostructure and the amino acid sequence as set forth in 10 PDB ID NO: 1IRE,
- (o) specifying, based on the inferred stereostructure, the region which forms a cavity through which a substrate passes from the outside of the enzyme toward the active center, or a product passes from the active center to the 15 outside of the enzyme,
- (p) specifying, among the amino acid residues constituting the above-specified region, the amino acid residues whose alteration leads to a change in the cavity size and further controls the easiness or difficulty in 20 passing of the substrate/product, and
- (q) performing substitution, insertion or deletion at one or more of the amino acid residues specified in the above (p).

25 61. A method for modifying an enzyme having the

nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, substrate specificity,  $V_{max}$ ,  $K_m$ , thermal stability, stability against the substrate and stability against the product, by 5 specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

10 (r) inferring the stereostructure of the enzyme having the nitrile hydratase activity before modification by carrying out an alignment based on the nitrile hydratase stereostructure and the amino acid sequence as set forth in PDB ID NO: 1IRE,

15 (s) specifying, based on the stereostructure inferred, the four amino acid residues such as the amino acid residues which correspond to the 89<sup>th</sup> amino acid glutamine (A89Q) and the 165<sup>th</sup> amino acid glutamic acid (A165E) as counted from the N-terminal in Chain A, and the amino acid residues which correspond to the 37<sup>th</sup> amino acid phenylalanine (B37F) and 20 the 48<sup>th</sup> amino acid leucine (B48L) as counted from the N-terminal in Chain B in the nitrile hydratase stereostructure as set forth in PDB ID NO: 1IRE,

(t) specifying the amino acid residues which effect a change in at least one of d1 to d3, when the shortest 25 distance between the heavy atoms of the amino acid residue

corresponding to A165E and of the amino acid residue  
corresponding to B48L is designated as d1; the shortest  
distance between the heavy atoms of the amino acid residue  
corresponding to A89Q and of the amino acid residue

5 corresponding to B48L as d2; and the shortest distance  
between the heavy atoms of the amino acid residue  
corresponding to B37F and of the amino acid residue  
corresponding to B48L as d3, and

10 (u) performing substitution, insertion or deletion at  
one or more sites of the specified amino acid residues.

62. The method for modification according to claim 61,  
wherein the step of (t) is replaced by the following step  
(t'):

15 (t') specifying the amino acid residues which effect a  
change in at least one of d1 to d5, when the shortest  
distance between the heavy atoms of the amino acid residue  
corresponding to A165E and of the amino acid residue  
corresponding to B48L is designated as d1; the shortest  
20 distance between the heavy atoms of the amino acid residue  
corresponding to A89Q and of the amino acid residue  
corresponding to B48L as d2; the shortest distance between  
the heavy atoms of the amino acid residue corresponding to  
B37F and of the amino acid residue corresponding to B48L is  
25 designated as d3; the shortest distance between the heavy

atoms of the amino acid residue corresponding to A165E and of the amino acid residue corresponding to B37F as d4; and the shortest distance between the heavy atoms of the amino acid residue corresponding to A89Q and of the amino acid residue corresponding to B37F as d5.

63. The method for modification according to any one of claims 56 to 62, wherein the enzyme having the nitrile hydratase activity before modification comprises the two 10 polypeptides [A] and [B] of the following:

[A] a polypeptide consisting of an amino acid sequence which shows homology of at least 40% with the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing, and

15 [B] a polypeptide consisting of an amino acid sequence which shows homology of at least 25% with the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing.

20 64. The method for modification according to claim 63, characterized in that the polypeptide of [A] is the polypeptide of the following [C], and the polypeptide of [B] is the polypeptide of the following [D]:

25 [C] a polypeptide consisting of any amino acid sequence selected from the amino acid sequence as set forth in SEQ ID

NO: 98 in the Sequence Listing; the amino acid sequence in which substitution, insertion or deletion has been implemented at one or more sites in the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing; and

5 the amino acid sequence in which at least one amino acid of the 6<sup>th</sup>, 19<sup>th</sup>, 38<sup>th</sup>, 77<sup>th</sup>, 90<sup>th</sup>, 102<sup>nd</sup>, 106<sup>th</sup>, 126<sup>th</sup>, 130<sup>th</sup>, 142<sup>nd</sup>, 146<sup>th</sup>, 187<sup>th</sup>, 194<sup>th</sup> and 203<sup>rd</sup> amino acids in the amino acid sequence as set forth in SEQ ID NO: 98 in the Sequence Listing is substituted by another amino acid, and

10 [D] a polypeptide consisting of any amino acid sequence selected from the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing; the amino acid sequence in which substitution, insertion or deletion has been implemented at one or more sites in the amino acid sequence

15 as set forth in SEQ ID NO: 99 in the Sequence Listing; and the amino acid sequence in which at least one amino acid of the 20<sup>th</sup>, 21<sup>st</sup>, 108<sup>th</sup>, 200<sup>th</sup> and 212<sup>th</sup> amino acids in the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing is substituted by another amino acid.

20

65. The method for modification according to claim 63, wherein the polypeptide of [A] is the polypeptide of the following [E], and the polypeptide of [B] is the polypeptide of the following [F]:

25 [E] a polypeptide consisting of an amino acid sequence

showing homology with the amino acid sequence which is encoded by the open reading frame (ORF) composed of from the 704<sup>th</sup> to 1315<sup>th</sup> of the base sequence as set forth in SEQ ID NO: 104 in the Sequence Listing, and

5 [F] a polypeptide consisting of an amino acid sequence showing homology with the amino acid sequence which is encoded by the ORF composed of from the 1<sup>st</sup> to 680<sup>th</sup> of the base sequence as set forth in SEQ ID NO: 104 in the Sequence Listing.

10

66. A method for modifying an enzyme having the nitrile hydratase activity, which comprises changing one or more properties selected from the enzymatic activity, substrate specificity,  $V_{max}$ ,  $K_m$ , thermal stability, stability 15 against the substrate and stability against the product, by specifying certain amino acid residues and performing substitution, insertion or deletion at one or more sites of the specified amino acid residues according to the following procedure:

20 (d') aligning the amino acid sequence of the enzyme having the nitrile hydratase activity before modification, with the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing,

(e') specifying, based on the results of the alignment, 25 the amino acid residues corresponding to the 48<sup>th</sup> and 51<sup>st</sup> in

the amino acid sequence as set forth in SEQ ID NO: 99 in the Sequence Listing, and

(f') performing substitution, insertion or deletion at one or more sites of the specified amino acid residues,

5 wherein among the two polypeptides constituting the enzyme having the nitrile hydratase activity before modification, one is the polypeptide of [E] according to claim 65 and the other is the polypeptide of [F] according to claim 65.

10 67. The method for modification according to claim 63, wherein the polypeptide of [A] is the polypeptide of the following [G] :

[G] a polypeptide containing the region as represented by the amino acid sequence  $X_1CXLC_1SC_2X_2X_3X_4X_5$  (wherein C 15 corresponds to cysteine, X to serine or threonine, L to leucine,  $C_1$  to cysteine sulfenic acid (cysteine sulfenic acid-3-sulfinoalanine), S to serine, and  $C_2$  to cysteine sulfenic acid (cysteine sulfenic acid-S-hydroxy-cysteine); and  $X_1$ ,  $X_2$ ,  $X_3$ ,  $X_4$  and  $X_5$  represent arbitrary amino acid, 20 respectively).

68. The method for modification according to claim 67, wherein  $X_1$  is valine,  $X_4$  is tryptophan, and  $X_5$  is praline.

25 69. The method for modification according to claim 68,

wherein  $X_2$  is tyrosine and  $X_3$  is praline.

70. The method for modification according to any one of claims 67 to 69, wherein bonding with a metal atom is 5 located in the region represented by  $X_1CXLC_1SC_2X_2X_3X_4X_5$ .

71. The method for modification according to claim 70, wherein the metal atom is a cobalt atom.

10 72. A modified enzyme obtained by the method for modification according to any one of claims 56 to 71.

73. The gene encoding the modified enzyme according to claim 72.

15 74. A plasmid containing the gene according to claim 73.

75. A transformant obtained by transformation of a 20 microorganism with the gene according to claim 73 or the plasmid according to claim 74.

76. A method for production of a modified enzyme, comprising the step of recovering a modified enzyme from a 25 culture obtained from cultivating the transformant according

to claim 75, the cultivated cells or a product obtained from the processing of the culture or the cultivated cells.

77. A method for production of an amide compound,  
5 comprising the step of bringing the modified enzyme that is obtained from a culture obtained from cultivating the transformant according to claim 75, the cultivated cells or a product obtained from the processing of the culture or the cultivated cells, or the method for production according to  
10 claim 76, into contact with a nitrile compound in a solvent to convert the nitrile compound to a corresponding amide compound.